

## AMENDMENTS TO THE CLAIMS

This listing of claims will replace prior versions and listings of claims in the application:

### Listing of claims:

1. (Original) A fluorogenic PHEX substrate comprising  
a peptide unit;  
a fluorophore unit capable of conferring fluorescence on said substrate attached to an amino acid residue at a first end of the peptide unit; and  
a quencher unit capable of providing intramolecular quenching of said fluorescence attached to an amino acid residue at a second end of the peptide unit;  
the peptide unit having at least 6 amino acids residues including a sequence P<sub>2</sub>-P<sub>1</sub>-P<sub>1'</sub>-P<sub>2'</sub> of 4 amino acid residues at positions P<sub>2</sub>, P<sub>1</sub>, P<sub>1'</sub> and P<sub>2'</sub> of the peptide unit, respectively; the amino acid residue at position P<sub>2</sub> being any amino acid residue; the amino acid residue at position P<sub>1</sub> being any amino acid residue except an isoleucine, a valine, or a histidine residue; the amino acid residue at position P<sub>1'</sub> being an acidic amino acid residue selected from the group consisting of a glutamic acid residue and an aspartic acid residue, and being located at least 2 amino acid residues distal to both the fluorophore and the quencher units; the amino acid residue at position P<sub>2'</sub> being any amino acid residue except a leucine, a proline or a glycine residue, with the proviso that said peptide unit does not have the sequence as set forth in SEQ ID NO:1.
2. (Original) A fluorogenic PHEX substrate as recited in claim 1, wherein said amino acid residue at position P<sub>1'</sub> is aspartic acid.
3. (Currently amended) A fluorogenic PHEX substrate as recited in claim 1 or claim 2, wherein said amino acid residue at position P<sub>2'</sub> is selected from the group consisting of a hydrophobic, an acidic and a polar amino acid residues.
4. (Currently amended) A fluorogenic PHEX substrate as recited in any one of claims 1 and 2 claim 1, wherein said amino acid residue at position P<sub>2'</sub> is selected from the group consisting of an asparagine, a glutamine, a methionine, an alanine, a valine, a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine, and an isoleucine residues.

5. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 1 and 2~~ claim 1, wherein said amino acid residue at position P<sub>2'</sub> is selected from the group consisting of a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine and an isoleucine residues.
6. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 1 and 2~~ claim 1, wherein said amino acid residue at position P<sub>2</sub> is not an arginine, a lysine, an asparagine or a glutamine residue.
7. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 1 to 6~~ claim 1, wherein said P<sub>2</sub>-P<sub>1</sub>-P<sub>1'</sub>-P<sub>2'</sub> is as set forth in SEQ ID NO:2.
8. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 1 to 7~~ claim 1, wherein the fluorophore unit is Abz and the quencher unit is Dnp, and wherein Dnp is attached to a lysine residue.
9. (Original) A fluorogenic PHEX substrate having the chemical structure Abz-(SEQ ID NO:3)-Dnp.
10. (Original) A method for identifying a PHEX modulator comprising contacting a candidate compound with PHEX in the presence of a PHEX substrate, said substrate including a peptide unit of at least 6 amino acids residues including a sequence P<sub>2</sub>-P<sub>1</sub>-P<sub>1'</sub>-P<sub>2'</sub> of 4 amino acid residues at positions P<sub>2</sub>, P<sub>1</sub>, P<sub>1'</sub> and P<sub>2'</sub> of the peptide unit, respectively; the amino acid residue at position P<sub>2</sub> being any amino acid residue; the amino acid residue at position P<sub>1</sub> being any amino acid residue except an isoleucine, a valine, or a histidine residue; the amino acid residue at position P<sub>1'</sub> being an acidic amino acid residue selected from the group consisting of a glutamic acid and an aspartic acid residue; the amino acid residue at position P<sub>2'</sub> being any amino acid residue except a leucine, a proline or a glycine residue, with the proviso that said peptide does not have the sequence as set forth in SEQ ID NO:1;  
detecting a product resulting of the PHEX enzymatic activity on said substrate; and wherein a difference in the amount of said product detected in the presence of said candidate compound as compared to that in the absence thereof is an indication that said candidate compound modulates PHEX.

11. (Original) A method as recited in claim 10, wherein said PHEX substrate further comprises a fluorophore unit capable of conferring fluorescence on said substrate, said fluorophore unit being attached to an amino acid residue at a first end of the peptide unit, and a quencher unit capable of providing intramolecular quenching of said fluorescence, said quencher unit being attached to an amino acid residue at a second end of the peptide unit, wherein  $P_1'$  is located at least 2 amino acid residues distal to both the fluorophore unit and the quencher unit and wherein the product is detected through a modulation of fluorescence.
12. (Currently amended) A method as recited in claim 10 or 11, wherein said amino acid residue at position  $P_1'$  is aspartic acid.
13. (Currently amended) A method as recited in ~~any one of claims 10 to 12~~ claim 10, wherein said amino acid residue at position  $P_2'$  is selected from the group consisting of a hydrophobic, an acidic and a polar amino acid residues.
14. (Currently amended) A method as recited in ~~any one of claims 10 to 12~~ claim 10, wherein said amino acid residue at position  $P_2'$  is selected from the group consisting of an asparagine, a glutamine, a methionine, an alanine, a valine, a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine, and an isoleucine residues.
15. (Currently amended) A method as recited in ~~any one of claims 10 to 12~~ claim 10, wherein said amino acid residue at position  $P_2'$  is selected from the group consisting of a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine and an isoleucine residues.
16. (Currently amended) A method as recited in ~~any one of claims 10 to 12~~ claim 10, wherein said amino acid residue at position  $P_2$  is not an arginine, a lysine, an asparagine or a glutamine residue.
17. (Original) A method as recited in claim 10, wherein  $P_2-P_1-P_1'-P_2'$  is as set forth in SEQ ID NO:2.
18. (Original) A method as recited in claim 11, wherein the fluorophore unit is Abz and the quencher unit is Dnp, and wherein Dnp is attached to a lysine residue.
19. (Original) A method as recited in claim 10, wherein the PHEX substrate has the chemical structure Abz-(SEQ ID NO:3)-Dnp.

20. (Currently amended) A method as recited in ~~any one of claims 10 to 19~~ claim 10, wherein the modulator is an inhibitor, and wherein a lower amount of said product detected in the presence of said candidate compound as compared to that in the absence thereof is an indication that said candidate compound inhibits PHEX.

21. (Original) A method for determining the presence and/or concentration of PHEX in a sample comprising

contacting said sample with a PHEX peptide substrate, said substrate including a peptide unit of at least 6 amino acids residues including a sequence  $P_2-P_1-P_1'-P_2'$  of 4 amino acid residues at positions  $P_2$ ,  $P_1$ ,  $P_1'$  and  $P_2'$  of the peptide unit, respectively; the amino acid residue at position  $P_2$  being any amino acid residue; the amino acid residue at position  $P_1$  being any amino acid residue except an isoleucine, a valine, or a histidine residue; the amino acid residue at position  $P_1'$  being an acidic amino acid residue selected from the group consisting of a glutamic acid and an aspartic acid residue; the amino acid residue at position  $P_2'$  being any amino acid residue except a leucine, a proline or a glycine residue, with the proviso that said peptide does not have the sequence as set forth in SEQ ID NO:1;

assessing the presence and/or concentration of a product resulting of the PHEX enzymatic activity on said substrate; and

wherein the presence and/or concentration of said product can be correlated to the presence/concentration of PHEX in the sample.

22. (Original) A method as recited in claim 21, wherein said PHEX substrate further comprises a fluorophore unit capable of conferring fluorescence on said substrate, said fluorophore unit being attached to an amino acid residue at a first end of the peptide unit; and a quencher unit capable of providing intramolecular quenching of said fluorescence, said quencher unit being attached to an amino acid residue at a second end of the peptide unit, wherein  $P_1'$  is located at least 2 amino acid residues distal to both the fluorophore unit and the quencher unit and wherein the product is detected through a modulation of fluorescence.

23. (Currently amended) A method as recited in claim 21-~~of 22~~, wherein said amino acid residue at position  $P_1'$  is aspartic acid.

24. (Currently amended) A method as recited in ~~any one of claims 21 to 23~~ claim 21, wherein said amino acid residue at position P<sub>2'</sub> is selected from the group consisting of a hydrophobic, an acidic and a polar amino acid residues.
25. (Currently amended) A method as recited in ~~any one of claims 21 to 23~~ claim 21, wherein said amino acid residue at position P<sub>2'</sub> is selected from the group consisting of an asparagine, a glutamine, a methionine, an alanine, a valine, a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine, and an isoleucine residues.
26. (Currently amended) A method as recited in ~~any one of claims 21 to 23~~ claim 21, wherein said amino acid residue at position P<sub>2'</sub> is selected from the group consisting of a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine and an isoleucine residues.
27. (Currently amended) A method as recited in ~~any one of claims 21 to 26~~ claim 21, wherein said amino acid residue at position P<sub>2</sub> is not an arginine, a lysine, an asparagine or a glutamine residue.
28. (Currently amended) A method as recited in claim 21-~~or~~ 22, wherein P<sub>2</sub>-P<sub>1</sub>-P<sub>1'</sub>-P<sub>2'</sub> is as set forth in SEQ ID NO:2.
29. (Original) A method as recited in claim 22, wherein the fluorophore unit is Abz and the quencher unit is Dnp, and wherein Dnp is attached to a lysine residue.
30. (Original) A method as recited in claim 21, wherein the PHEX substrate has the chemical structure Abz-(SEQ ID NO:3)-Dnp.
31. (Original) A fluorogenic PHEX substrate comprising  
a peptide unit;  
a fluorophore unit capable of conferring fluorescence on said substrate, said fluorophore being attached to an amino acid residue at a first end of the peptide unit; and  
a quencher unit capable of providing intramolecular quenching of said fluorescence, said quencher being attached to an amino acid residue at a second end of the peptide unit;  
the peptide unit comprising the sequence P<sub>3</sub>-P<sub>2</sub>-P<sub>1</sub>-P<sub>1'</sub>-P<sub>2'</sub>-P<sub>3'</sub> of amino acid residues at positions P<sub>3</sub>, P<sub>2</sub>, P<sub>1</sub>, P<sub>1'</sub>, P<sub>2'</sub>, and P<sub>3'</sub> of the peptide unit, respectively; the amino acid residue at position P<sub>2</sub> being any amino acid residue; the amino acid residue at position P<sub>1</sub> being any amino acid residue except an isoleucine, a valine, or a histidine residue; the amino acid

residue at position P<sub>1</sub>' being an acidic amino acid residue selected from the group consisting of a glutamic acid residue and an aspartic acid residue; the amino acid residue at position P<sub>2</sub>' being any amino acid residue except a leucine, a proline or a glycine residue, with the proviso that said peptide unit does not have the sequence as set forth in SEQ ID NO:1.

32. (Original) A fluorogenic PHEX substrate as recited in claim 31, wherein said amino acid residue at position P<sub>1</sub>' is aspartic acid.

33. (Currently amended) A fluorogenic PHEX substrate as recited in ~~claim 31~~ or ~~claim 32~~, wherein said amino acid residue at position P<sub>2</sub>' is selected from the group consisting of a hydrophobic, an acidic and a polar amino acid residues.

34. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 31 and 2~~ claim 31, wherein said amino acid residue at position P<sub>2</sub>' is selected from the group consisting of an asparagine, a glutamine, a methionine, an alanine, a valine, a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine, and an isoleucine residues.

35. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 31 and 32~~ claim 31, wherein said amino acid residue at position P<sub>2</sub>' is selected from the group consisting of a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine and an isoleucine residues.

36. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 31 to 35~~ claim 31, wherein said amino acid residue at position P<sub>2</sub> is not an arginine, a lysine, an asparagine or a glutamine residue.

37. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 31 to 36~~ claim 31, wherein said P<sub>2</sub>-P<sub>1</sub>-P<sub>1</sub>'-P<sub>2</sub>' is as set forth in SEQ ID NO:2.

38. (Currently amended) A fluorogenic PHEX substrate as recited in ~~any one of claims 31 to 37~~ claim 31, wherein the fluorophore unit is Abz and the quencher unit is Dnp, and wherein Dnp is attached to a lysine residue.

39. (Original) A method for identifying a PHEX modulator comprising contacting a candidate compound with PHEX in the presence of the fluorogenic PHEX substrate of claim 31;

detecting a product resulting of the PHEX enzymatic activity on said substrate; and wherein a difference in the amount of said product detected in the presence of said candidate compound as compared to that in the absence thereof is an indication that said candidate compound modulates PHEX.

40. (Original) A method for determining the presence and/or concentration of PHEX in a sample comprising contacting said sample with the fluorogenic PHEX substrate of claim 31; detecting a product resulting of the PHEX enzymatic activity on said substrate; and wherein a difference in the amount of said product detected in the presence of said candidate compound as compared to that in the absence thereof is an indication that said candidate compound modulates PHEX.

41. (Currently amended) A method as recited in claim 39-~~or~~40, wherein said amino acid residue at position  $P_1'$  is aspartic acid.

42. (Currently amended) A method as recited in ~~any one of claims 39 to 41~~ claim 39, wherein said amino acid residue at position  $P_2'$  is selected from the group consisting of a hydrophobic, an acidic and a polar amino acid residues.

43. (Currently amended) A method as recited in ~~any one of claims 39 to 41~~ claim 39, wherein said amino acid residue at position  $P_2'$  is selected from the group consisting of an asparagine, a glutamine, a methionine, an alanine, a valine, a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine, and an isoleucine residues.

44. (Currently amended) A method as recited in ~~any one of claims 39 to 41~~ claim 39, wherein said amino acid residue at position  $P_2'$  is selected from the group consisting of a tryptophan, a threonine, a serine, a tyrosine, a phenylalanine and an isoleucine residues.

45. (Currently amended) A method as recited in ~~any one of claims 39 to 44~~ claim 39, wherein said amino acid residue at position  $P_2$  is not an arginine, a lysine, an asparagine or a glutamine residue.

46. (Currently amended) A method as recited in claim 39-~~or~~40, wherein  $P_2-P_1-P_1'-P_2'$  is as set forth in SEQ ID NO:2.

47. (Currently amended) A method as recited in ~~any one of claims 39 to 46~~ claim 39, wherein the fluorophore unit is attached to P<sub>3</sub> and the quencher unit is attached to P<sub>3</sub>'.

48. (Original) A method as recited in claim 47 wherein the fluorophore unit is Abz, the quencher unit is Dnp, and P<sub>3</sub>' is a lysine residue.

**AMENDMENTS TO THE SEQUENCE LISTING**

An enclosed substitute sequence listing will replace the prior version of the sequence listing in the application.